abcam

ab214168

Anti-ERK1 antibody [Y72] - BSA and Azide free

Recombinant | RabMAb | KO Validated

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Constituents: PBS
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Carrier free	Yes
Clone number	Y72
Purification technique	Affinity purification Protein A
Specificity	This antibody recognises ERK1.The antibody does not cross-react with other MAP kinases.
Epitope	ab214168 reacts with an epitope located in the N terminal region of ERK1.
Concentration	1.042 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IHC-P

Tested

Species	Human
Dilution info	-
Notes	-

Expected

Species	Mouse
Dilution info	Use at an assay dependent concentration.
Notes	-

Not recommended

Species	Synthetic peptide - Human
Dilution info	-
Notes	-

IP

Tested

Species	Human
Dilution info	-
Notes	-

Expected

Species	Mouse
Dilution info	Use at an assay dependent concentration.
Notes	-

Not recommended

Species	Synthetic peptide - Human
Dilution info	-
Notes	-

WB

Tested

Species	Mouse
Dilution info	-
Notes	Can be blocked with ab204281.

Species	Human
Dilution info	-
Notes	Can be blocked with ab204281.

Species	Synthetic peptide - Human
Dilution info	-
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	-
Notes	This product gave a positive signal in HAP1 cells fixed with 4% formaldehyde (10 min) and 100% methanol (5 min).

Expected

Species	Mouse
Dilution info	Use at an assay dependent concentration.
Notes	-

Not recommended

Species	Synthetic peptide - Human
Dilution info	-
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	-
Notes	ab199376 - Rabbit monoclonal IgG, is suitable for use as an isotype control.

Expected

Species	Mouse
Dilution info	Use at an assay dependent concentration.
Notes	-

Not recommended

Species	Synthetic peptide - Human
Dilution info	-
Notes	-

Target data

See full target information MAPK3

Function

Serine/threonine kinase which acts as an essential component of the MAP kinase signal transduction pathway (PubMed:34497368). MAPK1/ERK2 and MAPK3/ERK1 are the 2 MAPKs which play an important role in the MAPK/ERK cascade. They participate also in a signaling cascade initiated by activated KIT and KITLG/SCF. Depending on the cellular context, the MAPK/ERK cascade mediates diverse biological functions such as cell growth, adhesion, survival and differentiation through the regulation of transcription, translation, cytoskeletal rearrangements. The MAPK/ERK cascade also plays a role in initiation and regulation of meiosis, mitosis, and postmitotic functions in differentiated cells by phosphorylating a number of transcription factors. About 160 substrates have already been discovered for ERKs. Many of these substrates are localized in the nucleus, and seem to participate in the regulation of transcription upon stimulation. However, other substrates are found in the cytosol as well as in other cellular organelles, and those are responsible for processes such as translation, mitosis and apoptosis. Moreover, the MAPK/ERK cascade is also involved in the regulation of the endosomal dynamics, including lysosome processing and endosome cycling through the perinuclear recycling compartment (PNRC); as well as in the fragmentation of the Golgi apparatus during mitosis. The substrates include transcription factors (such as ATF2, BCL6, ELK1, ERF, FOS, HSF4 or SPZ1),

cytoskeletal elements (such as CANX, CTTN, GJA1, MAP2, MAPT, PXN, SORBS3 or STMN1), regulators of apoptosis (such as BAD, BTG2, CASP9, DAPK1, IER3, MCL1 or PPARG), regulators of translation (such as EIF4EBP1) and a variety of other signaling-related molecules (like ARHGEF2, DEPTOR, FRS2 or GRB10) (PubMed:35216969). Protein kinases (such as RAF1, RPS6KA1/RSK1, RPS6KA3/RSK2, RPS6KA2/RSK3, RPS6KA6/RSK4, SYK, MKNK1/MNK1, MKNK2/MNK2, RPS6KA5/MSK1, RPS6KA4/MSK2, MAPKAPK3 or MAPKAPK5) and phosphatases (such as DUSP1, DUSP4, DUSP6 or DUSP16) are other substrates which enable the propagation the MAPK/ERK signal to additional cytosolic and nuclear targets, thereby extending the specificity of the cascade. Phosphorylates GJA1 at 'Ser-279' and 'Ser-282' resulting in an increase in GJA1 ubiquitination and ultimately lysosomal degradation (By similarity).

Storage

Shipped at conditions	Blue Ice
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	+4°C
Storage information	Do Not Freeze

Notes

ab214168 is the carrier-free version of ab32537.

Patented technology
Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

What are the advantages of a recombinant monoclonal antibody?
This product is a recombinant monoclonal antibody, which offers several advantages including:

- High batch-to-batch consistency and reproducibility
- Improved sensitivity and specificity
- Long-term security of supply
- Animal-free batch production

For more information, read more on recombinant antibodies.

Species reactivity
Rat: We have preliminary internal testing data to indicate this antibody may not react with this species.
Please contact us for more information.

Conjugation ready
Our carrier-free antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications.

Use our conjugation kits for antibody conjugates that are ready-to-use in as little as 20 minutes with 1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold.

Compatibility
This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc.

Supplementary info

This supplementary information is collated from multiple sources and compiled automatically.

Activity summary	ERK1 also known as MAPK3 is an extracellular signal-regulated kinase involved in transmitting signals from the cell surface to the nucleus. This protein has a molecular mass of about 44 kDa. ERK1 expresses in various tissue types with higher expression in the brain heart and skeletal muscle. Researchers often study ERK1 in the context of its role in cellular signaling due to its involvement in critical regulatory functions.
Biological function summary	ERK1 plays a significant role in cell cycle regulation differentiation and proliferation. It forms part of the MAPK signaling cascade becoming activated through a phosphorylation event. In its activated form ERK1 translocates to the nucleus where it phosphorylates target substrates. ERK1 often functions in conjunction with its homolog ERK2 to mediate these cellular processes marking it as an essential player in growth factor signaling.
Pathways	ERK1 functions primarily within the MAPK/ERK signaling pathway a major conduit for transmitting proliferative signals from growth factor receptors. ERK1 interacts with proteins like MEK1/2 which phosphorylate and activate ERK1 in response to extracellular stimuli. Another critical pathway involving ERK1 is the Ras-Raf-MEK-ERK cascade which regulates various cellular outcomes. This connection to the Ras family highlights its importance in signal transduction and reinforces its position in critical cellular processes.
Associated diseases and disorders	Aberrant activation of ERK1 connects to diseases such as cancer and cardiovascular disorders. In cancer the dysregulation of the MAPK/ERK pathway often through mutations affecting Ras or Raf proteins leads to uncontrolled cell proliferation. ERK1's involvement in cardiovascular diseases links to its role in hypertrophic signaling in cardiac cells where altered ERK1 activity can contribute to pathological cardiac remodeling. Understanding these interactions can aid in developing therapeutic strategies targeting the MAPK/ERK signaling pathway.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

We do not recommend this combination. It is not covered by our product promise.

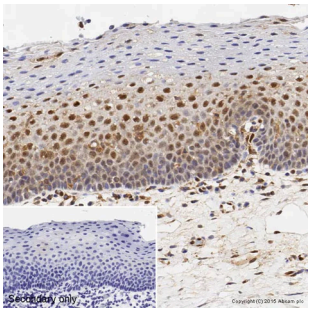
We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

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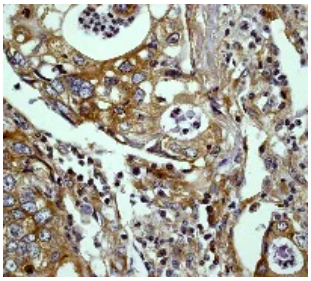
17 product images



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-ERK1 antibody [Y72] - BSA and Azide free (ab214168)

ERK1 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-ERK1 antibody

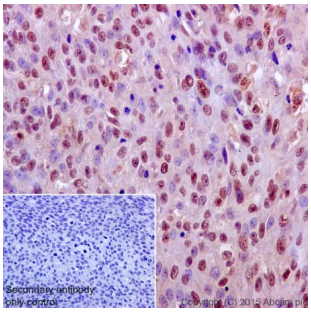
IHC image of ab32537 staining ERK1 in Human tonsil formalin fixed paraffin embedded tissue sections, performed on a Leica Bond. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20 mins. The section was then incubated with ab32537, 2µg/ml dilution, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX. No primary antibody was used in the secondary only control (shown on the inset).
For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32537).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-ERK1 antibody [Y72] - BSA and Azide free (ab214168)

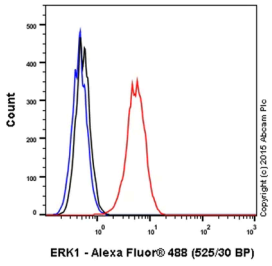
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human lung carcinoma tissue labelling ERK1 with unpurified ab32537.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32537).

Heat mediated antigen retrieval was performed before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-ERK1 antibody [Y72] - BSA and Azide free (ab214168)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human cervix carcinoma tissue labelling ERK1 with purified ab32537 at a dilution of 1/50. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. ab97051, a HRP-conjugated goat anti-rabbit IgG (H+L) was used as the secondary antibody (1/500). Negative control using PBS instead of primary antibody. Counterstained with hematoxylin.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32537).



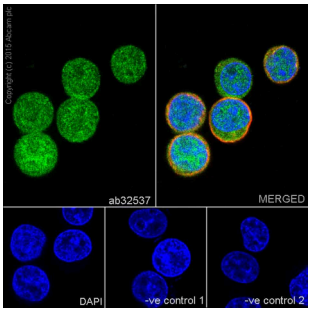
Flow Cytometry (Intracellular) - Anti-ERK1 antibody [Y72] - BSA and Azide free (ab214168)

ERK1 Flow Cytometry (Intracellular) staining using rabbit Anti-ERK1 antibody

Overlay histogram showing HeLa cells stained with ab32537 (red line). The cells were fixed with 4% formaldehyde (10 min) and then permeabilized with 0.1% PBS-Tween 20 for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab32537, 1/11312) for 30 min at 22°C. The secondary antibody used was Alexa Fluor® 488 goat anti-rabbit IgG (H&L) (ab150081) at 1/2000 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit IgG (monoclonal) (ab172730, 0.01µg/1x10^6 cells) used under the same conditions. Unlabelled sample (blue line) was also used as a control.
Acquisition of >5,000 events were collected using a 20mW Argon ion laser (488nm) and 525/30 bandpass filter.

This antibody gave a positive signal in HeLa cells fixed with 80% methanol (5 min)/permeabilized with 0.1% PBS-Tween 20 for 20 min used under the same conditions.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32537).



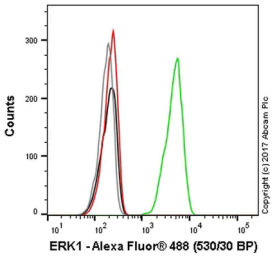
Immunocytochemistry/ Immunofluorescence - Anti-ERK1 antibody [Y72] - BSA and Azide free (ab214168)

ERK1 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-ERK1 antibody

Immunocytochemistry/Immunofluorescence analysis of Jurkat cells labelling ERK1 with purified ab32537 at a dilution of 1/100. Cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. ab150077, an Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/1000) was used as the secondary antibody. DAPI (blue) was used as the nuclear counterstain. ab7291, a mouse anti-tubulin (1/1000) and ab150120, an Alexa Fluor® 594-conjugated goat anti-mouse IgG (1/1000) were also used.
Control 1: primary antibody (1/100) and secondary antibody, ab150120, an Alexa Fluor® 594-conjugated goat anti-mouse IgG (1/1000).

Control 2: ab7291 (1/1000) and secondary antibody, ab150077, an Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/1000).

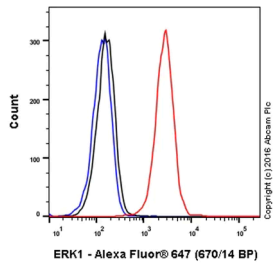
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32537).



Flow Cytometry (Intracellular) - Anti-ERK1 antibody [Y72] - BSA and Azide free (ab214168)

ERK1 Flow Cytometry (Intracellular) staining using rabbit Anti-ERK1 antibody

Overlay histogram showing HAP1 wildtype (green line) and HAP1-MAPK3 knockout cells (red line) stained with ab32537. The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Triton X-100 for 15 min. The cells were then incubated in 1x PBS / 10% normal goat serum to block non-specific protein-protein interactions followed by the antibody (ab32537, 1µg/ml) for 30 min at 22°C. The secondary antibody used was Alexa Fluor® 488 goat anti-rabbit IgG (H&L) (ab150081) at 1/2000 dilution for 30 min at 22°C. A Rabbit IgG isotype control antibody (ab172730) was used at the same concentration and conditions as the primary antibody (HAP1 wildtype - black line, HAP1-MAPK3 knockout - grey line). Unlabelled sample was also used as a control (this line is not shown for the purpose of simplicity). Acquisition of >5,000 events were collected using a 50 mW Blue laser (488nm) and 530/30 bandpass filter. This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32537).



Flow Cytometry (Intracellular) - Anti-ERK1 antibody [Y72] - BSA and Azide free (ab214168)

ERK1 Flow Cytometry (Intracellular) staining using rabbit Anti-ERK1 antibody

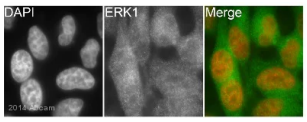
Clone Y72 (ab214168) has been successfully conjugated by Abcam. This image was generated using Anti-ERK1 antibody [Y72] (Alexa Fluor® 647). Please refer to ab190579 for protocol details.
Overlay histogram showing HeLa cells stained with ab190579 (red line). The cells were fixed with 4% formaldehyde and then permeabilized with 0.1% PBS-Triton X-100 for 15 min.

The cells were then incubated in 1x PBS / 10% normal goat serum to block non-specific protein-protein interactions followed by the antibody (ab190579, 1/5000 dilution) for 30 min at 22°C.

Isotype control antibody (black line) was Rabbit IgG (monoclonal) Alexa Fluor® 647 (ab199093) used at the same concentration and conditions as the primary antibody. Unlabelled sample (blue line) was also used as a control.

Acquisition of >5,000 events were collected using a 40 mW Red laser (640nm) and 670/14 bandpass filter.

This antibody gave a positive signal in HeLa cells fixed with 80% methanol (5 min), permeabilized with 0.1% PBS-Triton X-100 for 15 min used under the same conditions.

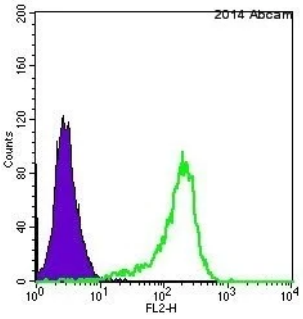


This image is courtesy of a customer review submitted by Kirk McManus.

Immunocytochemistry/ Immunofluorescence - Anti-ERK1 antibody [Y72] - BSA and Azide free (ab214168)

ERK1 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-ERK1 antibody

Unpurified ab32537 staining ERK1 in HeLa cells by ICC/IF (Immunocytochemistry/immunofluorescence). Cells were fixed with paraformaldehyde and permeabilized with 0.5% Triton X-100 in PBS. Samples were incubated with primary antibody (1/500 in PBS) for 1 hour at 22°C. ab150081, a goat anti rabbit Alexa Fluor® 488 (1/200) was used as the secondary antibody. Counterstained with DAPI. Cytoplasmic and nuclear staining shown.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32537).

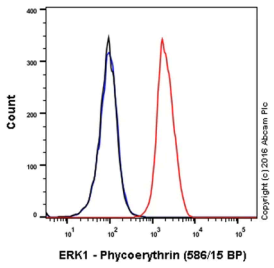


This image is courtesy of an anonymous customer review

Flow Cytometry (Intracellular) - Anti-ERK1 antibody [Y72] - BSA and Azide free (ab214168)

ERK1 Flow Cytometry (Intracellular) staining using rabbit Anti-ERK1 antibody

Unpurified ab32537 staining ERK1 (green) in HEK293 cells by intracellular flow cytometry. Cells were fixed with paraformaldehyde and permeabilized with 70% methanol. The sample was incubated with the primary antibody (1/40 in PBS + 0.2% BSA + 0.1% sodium azide) for 1 hour at 22°C. A phycoerythrin-conjugated goat anti-rabbit IgG (1/100) was used as the secondary antibody.
Gating Strategy: Live Cells. Purple plot represents isotype control.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32537).



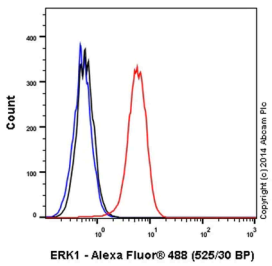
Flow Cytometry (Intracellular) - Anti-ERK1 antibody [Y72] - BSA and Azide free (ab214168)

ERK1 Flow Cytometry (Intracellular) staining using rabbit Anti-ERK1 antibody

Clone Y72 (ab214168) has been successfully conjugated by Abcam. This image was generated using Anti-ERK1 antibody [Y72] (PE). Please refer to ab210828 for protocol details.
Overlay histogram showing Jurkat cells stained with ab210828 (red line). The cells were fixed with 4% formaldehyde and then permeabilized with 90% methanol at -20°C for 15 min. The cells were then incubated in 1x PBS / 10% normal goat serum to block non-specific protein-protein interactions followed by the antibody (ab210828, 1/5000 dilution) for 30 min at 22°C.

Isotype control antibody (black line) was rabbit IgG (monoclonal) Phycoerythrin (ab209478) used at the same concentration and conditions as the primary antibody. Unlabelled sample (blue line) was also used as a control.

Acquisition of >5,000 events were collected using a 50mW Yellow/Green laser (561nm) and 586/15 bandpass filter.



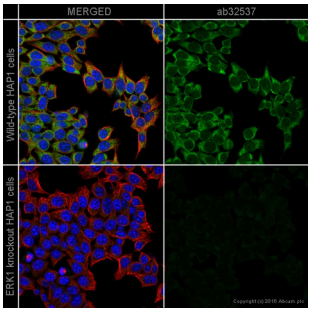
Flow Cytometry (Intracellular) - Anti-ERK1 antibody [Y72] - BSA and Azide free (ab214168)

ERK1 Flow Cytometry (Intracellular) staining using rabbit Anti-ERK1 antibody

Clone Y72 (ab214168) has been successfully conjugated by Abcam. This image was generated using Anti-ERK1 antibody [Y72] (Alexa Fluor® 488). Please refer to ab190200 for protocol details.
Overlay histogram showing HeLa cells stained with ab190200 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab190200, 1/500 dilution) for 30 min at 22°C. Isotype control antibody (black line) was rabbit IgG (monoclonal) Alexa Fluor® 488 used at the same concentration and conditions as the primary antibody. Unlabelled sample (blue line) was also used as a control.

Acquisition of >5,000 events were collected using a 20mW Argon ion laser (488nm) and 525/30 bandpass filter.

This antibody gave a positive signal in HeLa fixed with 4% formaldehyde (10 min)/permeabilized with 0.1% PBS-Tween for 20 min used under the same conditions.



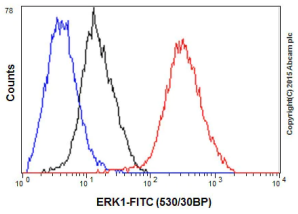
Immunocytochemistry/ Immunofluorescence - Anti-ERK1 antibody [Y72] - BSA and Azide free (ab214168)

ERK1 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-ERK1 antibody

ab32537 staining ERK1 in wild-type HAP1 cells (top panel) and ERK1 knockout HAP1 cells (bottom panel). The cells were fixed with 100% methanol (5min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated with ab32537 at 1/400 dilution and ab7291 at 1ug/ml concentration overnight at +4°C, followed by a further incubation at room temperature for 1h with a goat secondary antibody to Rabbit IgG (Alexa Fluor® 488) (ab150081) at 2 µg/ml (shown in green) and a goat secondary antibody to Mouse IgG (Alexa Fluor® 594) (ab150117) at 2ug/ml (shown in pseudo-color red). Nuclear DNA was labelled in blue with DAPI.
This product also gave a positive signal under the same testing conditions in HAP1 cells fixed with 4% formaldehyde (10 min).

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).

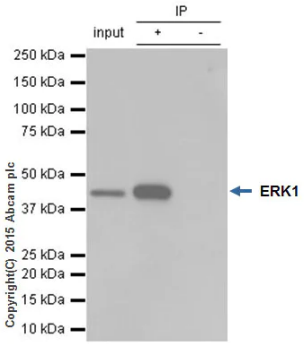
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32537).



Flow Cytometry (Intracellular) - Anti-ERK1 antibody [Y72] - BSA and Azide free (ab214168)

ERK1 Flow Cytometry (Intracellular) staining using rabbit Anti-ERK1 antibody

Intracellular Flow Cytometry analysis of Jurkat cells labelling ERK1 with purified ab32537 at a dilution of 1/30 (red). Cells were fixed with 4% paraformaldehyde. A FITC-conjugated goat anti-rabbit IgG (1/150) was used as the secondary antibody. Black - Isotype control, rabbit monoclonal IgG. Blue - Unlabelled control, cells without incubation with primary and secondary antibodies.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32537).



Immunoprecipitation - Anti-ERK1 antibody [Y72] - BSA and Azide free (ab214168)

ab32537 (purified) at a dilution of 1/20 immunoprecipitating ERK1 in Jurkat whole cell lysate.
Lane 1 (input): Jurkat whole cell lysate (10µg)

Lane 2 (+): ab32537 + Jurkat whole cell lysate.

Lane 3 (-): Rabbit monoclonal IgG (ab172730) instead of ab32537 in Jurkat whole cell lysate.

For western blotting, VeriBlot for IP Detection Reagent (HRP) (ab131366), was used for detection at 1/10,000 dilution.

Blocking buffer and concentration: 5% NFDM/TBST.

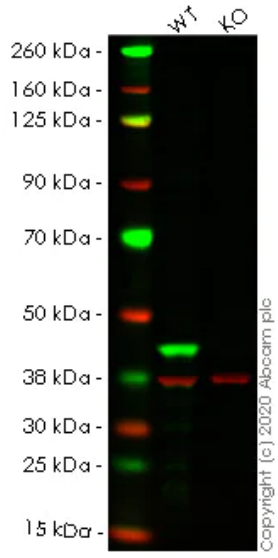
Diluting buffer and concentration: 5% NFDM /TBST.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab32537).

All lanes:
Immunoprecipitation - Anti-ERK1 antibody [Y72] (ab32537)

Predicted band size: 43 kDa

Observed band size: 44 kDa



Western blot - Anti-ERK1 antibody [Y72] - BSA and Azide free (ab214168)

ERK1 Western blot staining using rabbit Anti-ERK1 antibody

This data was developed using the same antibody clone in a different buffer formulation (ab32537).
Lanes 1- 2: Merged signal (red and green). Green - ab32537 observed at 43 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (ab8245) observed at 37 kDa.

ab32537 was shown to react with ERK1 in wild-type HEK-293T cells in western blot. Loss of signal was observed when knockout cell line ab266519 (knockout cell lysate ab257099) was used. Wild-type HEK-293T and MAPK3 knockout HEK-293T cell lysates were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. ab32537 and Anti-GAPDH antibody [6C5] - Loading Control (ab8245) overnight at 4°C at a 1 in 1000 dilution and a 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye®800CW) preadsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye®680RD) preadsorbed (ab216776) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.

All lanes:
Western blot - Anti-ERK1 antibody [Y72] (ab32537) at 1/1000 dilution

Lane 1:
Wild-type HEK-293T cell lysate at 20 µg

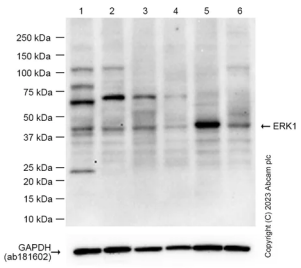
Lane 2:
MAPK3 knockout HEK-293T cell lysate at 20 µg

Lane 2:
Western blot - Human MAPK3 (ERK1) knockout HEK-293T cell line (ab266519)

Performed under reducing conditions.

Predicted band size: 43 kDa

Observed band size: 43 kDa



Western blot - Anti-ERK1 antibody [Y72] - BSA and Azide free (ab214168)

Blocking buffer and concentration: 5% NFDM/TBST. **Diluting buffer and concentration:** 5% NFDM/TBST. This blot was developed using a **high** sensitivity ECL substrate. ab181602 was used as a loading control. This data was developed using the same antibody clone in a different buffer formulation (ab32537).
All lanes:
Western blot - Anti-ERK1 antibody [Y72] (ab32537) at 1/1000 dilution

Lane 1:
Mouse brain lysate at 20 µg

Lane 2:
Mouse heart lysate at 20 µg

Lane 3:
Mouse kidney lysate at 20 µg

Lane 4:
Mouse spleen lysate at 20 µg

Lane 5:
Raw 264.7 (Mouse Abelson murine leukemia virus-induced tumor macrophage) whole cell lysate at 20 µg

Lane 6:
NIH/3T3 (Mouse embryonic fibroblast) whole cell lysate at 20 µg

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

Predicted band size: 43 kDa

Observed band size: 43 kDa

Exposure time: 180s

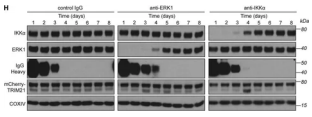


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Western blot - Anti-ERK1 antibody [Y72] - BSA and Azide free (ab214168)

ERK1 Western blot staining using rabbit Anti-ERK1 antibody

ERK1 western blot using anti-ERK1 antibody [Y72] - BSA and Azide free ab214168. Publication image and figure legend from Clift, D., McEwan, W. A., et al., 2017, Cell, PubMed 29153837.

ab214168 was used in this publication in western blot. This may not be the same as the application(s) guaranteed by Abcam. For a full list of applications guaranteed by Abcam for ab214168 please see the product overview.

Time Frame for Protein Depletion by Trim-Away, Related to Figure 5(A–F) NIH 3T3-mCherry-TRIM21 cells were taken up into the Neon Pipette Tip and either electroporated or not (mock). Percentage of dead cells was determined using the trypan blue exclusion assay (A). For long-term analysis of cellular behavior cells were imaged every 15 min for 70 h following electroporation (B-F). For the growth curves cell density was normalized to the initial density once cells had adhered (D). For adherence analysis the percentage of spread cells was quantified in each frame (B and C). Doubling times (E and F) were calculated taking the 5 h time point as reference, because cells were fully adhered then. Data from three independent experiments. Error bars show s.d. P values were calculated with Student’ s t test. See also Movie S4.(G and H) HEK293T-mCherry-TRIM21 cells were electroporated with either control IgG, anti-ERK1 or anti-IKKα antibodies and whole cell lysates harvested at the indicated times after electroporation for immunoblot.(I–M) NIH 3T3-mCherry-TRIM21 cells were electroporated with control IgG or anti-Pericentrin antibody (BD611815) and 3 hours later analyzed for Pericentrin (I and K) or Cdk5rap2 (J and L) localization or cell lysates were immunoblotted for the indicated proteins (M). Merge also shows DNA stained with Hoechst. Number of cells in brackets. Scale bars, 5 μm. Data from two independent experiments. P values were calculated with Fisher’ s exact (K) or Student’ s t test (L).

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